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TITLE: Vectors and methods for immunization or therapeutic protocols

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CLAIMS:

What is claimed is: .

1. A method for producing an immunostimulatory nucleic acid construct comprising at least one CpG-S motif and a nucleic acid encoding an antigen comprising:

determining CpG-N and CpG-S motifs present in a nucleic acid construct comprising at least one CpG-S motif and a nucleic acid encoding an antigen;

removing CpG-N motifs from the nucleic acid construct; and

optionally inserting CpG-S motifs into the nucleic acid construct, thereby producing said immunostimulatory nucleic acid construct that stimulates an immune response against the antigen,

wherein the CpG-N motifs comprise motifs selected from the group consisting of direct repeats of CpG dinucleotides, CCG trinuclectides, CGG trinucleotides, CCGG tetranucleotides, CGCG tetranucleotides, a combination thereof, and a poly-G motif, wherein the poly-G motif optionally contains at least four Gs in a row or two G trimers, and

wherein the CpG-S motifs comprise motifs having the formula 5'X.sub.1 CGX.sub.2 3' wherein C is unmethylated, at least one nucleotide separates consecutive CG dinucleotides in the motif, X.sub.1 is selected from the group consisting of adenine, guanine, and thymine and X.sub.2 is selected from the group consisting of cytosine, thymine, and adenine, and

wherein the CpG-N motifs are removed from non-essential regions of the nucleic acid construct and the CpG-S motifs are inserted into non-essential regions of the nucleic acid construct,

wherein the antigen is selected from the group consisting of a manmmalian

antigen, an avian antigen, an antigen from a pathogen that infects mammalian and avian subjects, wherein the pathogen is selected from the group consisting of a bacterium, a virus, a fungus and a parasite.

2. The method of claim 1, wherein the CpG-N motifs are removed by site-specific mutagenesis.

3. The method of claim 1, wherein the CpG-N motifs are selected from the group consisting of clusters of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetranucilcotides, CGCG tetranucleotides and a combination thereof.

4. The method of claim 1, wherein the nucleic acid construct is a plasmid.

5. The method of claim 1, wherein the nucleic acid construct is a viral vector.

6. The method of claim 1, wherein the CpG-S motifs in the immunostimulatory nucleic acid construct comprise a CpG motif having the formula:

5'X.sub.1 CGX.sub.2 3'

wherein at least one nucleotide separates consecutive CpGs, X.sub.1 is adenine, guanine, or thymine and X.sub.2 is cytosine, thymine, or adenine.

7. The method of claim 6, wherein the CpG-S motif is selected from the group consisting of GACGTT ,AGCGTT, AACGCT GTCGTT and AACGAT.

8. The method of claim 6, wherein the CpG-S motif comprises GTCGYT or TGACGTT.

9. The method of claim 6, wherein fie CpG-S motif comprises TGTCGYT.

10. The mdhod of claim 6, wherein the CpG-S motif comprises TCCATGTCGTTCCTGTCGTT (SEQ ID NO:1).

11. The method of claim 6, wherein the CpG-S motif comprises TCCTGACGTTCCTGACGTT (SEQ ID NO:2).

12. The method of claim 6, wherein the CpG-S motif comprises TCGTCGTTTTGTCGTTTGTCGTT (SEQ ID NO:3).

13. The method of claim 6, wherein the CpG-S motif comprises TCAACGTT.

14. The method of claim 1, wherein the antigen is a viral antigen.

15. The method of claim 14, wherein the viral antigen is from Hepatitis B virus (HBV).

16. The method of claim 15, wherein the viral antigen is HBV surface antigen.

17. The method of claim 1, wherein the nucleic acid construct further comprises regulatory sequences for expression of DNA in eukaryotic cells.

18. The method of claim 17, wherein the regulatory sequence is a promoter.

19. The method of claim 18, wherein the promoteris a viral promoter.

20. The method of claim 19, wherein the promoter is a CMV promoter.

21. The method of claim 18, wherein the promoter is insensitive to cytokine regulation.

22. The method of claim 18, wherein the promoter is cytokine sensitive.

23. The method of claim 18, wherein the promoter is a non-viral promoter.

24. The method of claim 18, wherein the promoter is a tissue- or cell-specific promoter.

25. The method of claim 24, wherein the cell specific promoter is operative in antigen-presenting cells.

26. The method of claim 25, wherein the promoter is a mammalian MHC I promoter.

27. A method for enhancing the iminunostimulatory effect of an antigen in a mammalian or avian subject, comprising

administering to the subject an effective amount of the immunostimulatory nucleic acid construct of claim 1 encoding the antigen,

wherein the antigen is selected from the group consisting of a mammalian antigen, an avian antigen, an antigen from a pathogen that infects mammalian and avian subjects, wherein the pathogen is selected from the group consisting of a bacterium, a virus, a fungus and a parasite.

28. The method of claim 1, wherein the antigen is a bacterial antigen.

29. The method of claim 1, wherein the antigen is derived from a parasite.

30. A method of eliciting an immune response against an antigen in a mammalian or avian subject comprising:

administering to the subject an effective amount of an antigen-encoding immunostimulatory nucleic acid construct comprising at least one CpG-S motif and produced by

determining CpGN and CpG-S motifs present in an antigen-encoding nucleic acid construct comprising at least one CpG-S motif; and

removing CpG-N motifs from the nucleic acid construct and

optionally inserting CpG-S motifs into the nucleic acid construct,

thereby eliciting an immune response against the antigen in the mammalian or avian subject,

wherein the CpG-N motifs comprise motifs selected from the group consisting of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetranucleotides, CGCG tetranucleotides, a combination thereof, and a poly-G motif, wherein the poly-G motif optionally contains at least four Gs in a row or two G trimers,

wherein the CpG-S motifs comprise motifs having the formula 5'X.sub.1 CGX.sub.2 3' wherein C is unmethylated, at least one nucleotide separates consecutive CG dinucleotides in the motif X.sub.1 is selected from the group consisting of adenine, guanine, and thymine and X.sub.2 is selected from the group consisting of cytosine, thymine; and adenine,

wherein the CpG-N motifs are removed from non-essential regions of the nucleic acid construct and the CpG-S motifs are inserted into non-essential regions of the nucleic acid construct.

31. The method of claim 30, wherein the nucleic acid construct further

comprises regulatory sequences for expression of DNA in eukaryotic cells.

32. The method of claim 31, wherein the regulatory sequence is a promoter.

33. The method of claim 32, wherein the promoter is a viral promoter.

34. The method of claim 33, wherein the promoter is a CMV promoter.

35. The method of claim 32, wherein the promoter is insensitive to cytokine regulation.

36. The method of claim 32, wherein the promoter is cytokine sensitive.

37. The method of claim 32, wherein the promoter is a non-viral promoter.

38. The method of claim 32, wherein the promoter is a tissue-specific promoter.

39. The method of claim 32, wherein the promoter is a cell-specific promoter.

40. The method of claim 39, wherein the cell-specific promoter is operative in antigen-presenting cells.

41. The method of claim 40, wherein the promoter is a mammalian MHC I promoter.

42. The method of claim 30, wherein the antigen is a viral antigen.

43. The method of claim 42, wherein the viral antigen is from Hepatitis B virus (HBV).

44. The method of claim 30, wherein the CpG-N motifs are selected from the group consisting of clusters of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetranucleotides, CGCG tetranucleotides and a combination thereof.

45. The method of claim 30, wherein the nucleic acid construct is a plasmid.

46. The method of claim 30, wherein the nucleic acid construct is a viral vector.

47. The method of claim 30, wherein the CpG-S motifs in the immunostimulatory nucleic acid construct comprise a CpG motif having the formula:

5'X.sub.1 CGX.sub.2 3'

wherein at least one nucleotide separates consecutive CpGs, X.sub.1 is adenine, guanine, or thymine and X.sub.2 is cytosine, thymine, or adenine.

48. The method of claim 47, wherein the CpG-S motif is selected from the group consisting of GACGTT, AGCGTT, AACGCT, GTCGTT and AACGAT.

49. The method of claim 47, wherein the CpG-S motif comprises GTCGYT or TGACGTT.

50. The method of claim 47, wherein the CpG-S motif comprises TGTCGYT.

51. The method of claim 47, wherein the CpG-S motif comprises TCCATGTCGTTCCTGTCGTT (SEQ ID NO:1).

52. The method of claim 47, wherein the CpG-S motif comprises TCCTGACGTTCCTGACGTT (SEQ ID NO:2).

53. The method of claim 47, wherein the CpG-S motif comprises TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:3).

54. The method of claim 47, wherein the CpG-S motif comprises TCAACGTT.

55. The method of claim 30, wherein the antigen is derived from a parasite.

56. The method of claim 30, further comprising administering an antigen to the subject.

57. The method of claim 56, wherein the antigen is administered to the subject essentially simultaneously with the immunostimulatory nucleic acid construct.

58. The method of claim 30, wherein the antigen is a bacterial antigen.

59. The method of claim 30, wherein the antigen is derived from a parasite.

60. A method for producing an immunostimulatory nucleic acid construct comprising at least one CpG-S motif and a nucleic acid encoding an antigen comprising:

determining CpG-N and CpG-S motifs present in a nucleic acid construct comprising at least one CpG-S motif;

removing CpG-N motifs from the nucleic acid construct; and

optionally inserting CpG-S motifs into the nucleic acid construct,

then inserting the nucleic acid encoding the antigen into the nucleic acid construct, thereby producing said immunostimulatory nucleic acid construct that stimulates an immune response against the antigen,

wherein the CpG-N motifs comprise motifs selected from the group consisting of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetranucleotides, CGCG tetranucleotides, a combination thereof, and a poly-G motif, wherein the poly-G motif optionally contains at least four Gs in a row or two G trimers, and

wherein the CpG-S motifs comprise motifs having the formula 5'X.sub.1 CGX.sub.2 3' wherein C is unmethylated, at least one nucleotide separates consecutive CG dinucleotides in the motif, X.sub.1 is selected from the group consisting of adenine, guanine, and thymine and X.sub.2 is selected from me group consisting of cytosine, thymine, and adenine, and

wherein the CpG-N motifs are removed from non-essential regions of the nucleic acid construct and the CpG-S motifs arc inserted into non-essential regions of the nucleic acid construct,

wherein the antigen is selected from the group consisting of a mammalian antigen, an avian antigen, an antigen from a pathogen that infects mammalian and avian subjects, wherein the pathogen is selected from the group consisting of a bacteriun, a virus, a fungus and a parasite.

61. The method of claim 60, wherein the CpG-N motifs are selected from the group consisting of clusters of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetmanucleotides, CGCG tetranucleotides and a combination thereof.

62. The methood of claim 60, wherein the nucleic acid construct is a plasmid.

63. The method of claim 60, wherein the nucleic acid construct is a viral vector.

64. The method of claim 60, wherein tie CpG-S motifs in the immunostimulatory nucleic acid construct comprise a CpG motif having the formula:

5'X.sub.1 CGX.sub.2 3'

wherein at least one nucleotide separates consecutive CpGs, X.sub.1 is adenine, guanine, or thymine and X.sub.2 is cytosine, thymine, or adenine.

65. The method of claim 64, wherein the CpG-S motif is selected from the group consisting of GACGTT, AGCGTT, AACGCT, GTCGTT and AACGAT.

66. the method of claim 64, wherein the CpG-S motif comprises GTCGYT or TGACGTT.

67. The method of claim 64, wherein the CpG-S motif comprises TGTCGYT.

68. The method of claim 64, wherein the CpG-S motif comprises TCCATGTCGTTCCTGTCGTT (SEQ ID: NO:1).

69. The method of claim 64, wherein the CpG-S motif comprises TCCTGACGTTCCTGACGTT (SEQ ID NO:2).

70. The method of claim 64, wherein the CpG-S motif comprises TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:3).

71. The method of claim 64, wherein the CpG-S motif comprises TCAACGTT.

72. The method of claim 60, wherein the antigen is a viral antigen.

73. The method of claim 72, wherein the viral antigen is firom Hepatitis B virus (HBV).

74. The method of claim 73, wherein the viral antigen is HBV surface antigen.

75. The method of claim 65, further comprising inserting to the nucleic acid construct regulatory sequences for expression of DNA in eukaryotic cells.

76. The method of claim 75, wherein the regulatory sequence is a promoter.

77. The method of claim 76, wherein the promoter is a viral promoter.

78. The method of claim 77, wherein the promoter is a CMV promoter.

79. The method of claim 76, wherein the promoter is a tissue- or cell-specific promoter.

80. The method of claim 79, wherein the cell specific promoter is operative in antigen-presenting cells.

81. The method of claim 80, wherein the promoter is a mammalian MHC I promoter.

82. The method of claim 60, wherein the antigen is a bacterial antigen.

83. The method of claim 60, wherein the antigen is derived from a parasite.

84. A method for enhancing the immunostimulatory effect of an antigen in a mammalian or avian subject, comprising

administering to the subject an effective amount of the immunostimulatory nucleic acid construct of claim 60 encoding the antigen,

wherein the antigen is selected from the group consisting of a mammalian antigen, an avian antigen, an antigen from a pathogen that infects mamalian and avian subjects, wherein the pathogen is selected from the group consisting of a bacterium, a virus, a fungus and a parasite.

85. A method of eliciting an immune response against an antigen in a mammalian or avian subject comprising:

administering to the subject an effective amount of an antigen-encoding immunostimulatory nucleic acid construct comprising at least one CpG-S motif and produced by

determining CpG-N and CpG-S motifs present in a nucleic acid construct comprising at least one CpG-S motif; and

removing CpG-N motifs from the nucleic acid construct,

optionally inserting CpG-S motifs into the nucleic acid construct, and

then inserting a nucleic acid encoding an antigen into the nucleic acid construct, thereby eliciting an immune response against the antigen in the mammalian or avian subject,

wherein the CpG-N motifs comprise motifs selected from the group consisting of direct repeats of CpG dinucleotides, CCG trinucleotides, CGG trinucleotides, CCGG tetranucleotides, CGCG tetranucleotides, a combination thereof, and a poly-G motif, wherein the poly-G motif optionally contains at least four Gs in a row or two G trimers,

wherein the CpG-S motifs comprise motifs having the formula 5'X.sub.1 CGX.sub.2 3' wherein C is unmethylated, at least one nucleotide separates consecutive CG dinucleotides in the motif, X.sub.1 is selected from the group consisting of adenine, guanine, and thymine and X.sub.2 is selected from the group consisting of cytosine, thymine, and adenine,

wherein the CpG-N motifs are removed from non-essential regions of the nucleic acid construct and the CpG-S motifs are inserted into non-essential regions of the nucleic acid construct.

86. The method of claim 85, further comprising inserting into the nucleic acid construct regulatory sequences for expression of DNA in eukaryotic cells.

87. The method of claim 86, wherein the regulatory sequence is a promoter.

88. The method of claim 87, wherein the promoter is a viral promoter.

89. The method of claim 87, wherein the promoter is a CMV promoter.

90. The method of claim 87, wherein the promoter is a tissue-specific promoter.

91. The method of claim 87, wherein the promoter is a cell-specific promoter.

92. The method of claim 91, wherein the cell-specific promoter is operative in autigen-presenting cells.

93. The method of claim 92, wherein the promoter is a mammalian MHC I promoter.

94. The method of claim 85, wherein the antigen is a viral antigen.

95. The method of claim 94, wherein the viral antigen is from Hepatitis B virus

(HBV).

96. The method of claim 85, wherein the CpG-N motifs are selected from the group consisting of clusters of direct repeats of CpG dinucieotides, CCG trinucleotides; CGG trinucleotides, CCGG tetranuclootides, CGCG tetranucleotides and a combination thereof.

97. The method of claim 85, wherein the nucleic acid construct is a plasmid.

98. The method of claim 85, wherein the nucleic acid construct is a viral vector.

99. The method of claim 85, wherein the CpG-S motifs in the itmmunostimulatory nucleic acid construct comprise a CpG motif having the formula:

5'X.sub.1 CGX.sub.2 3'

wherein at least one nucleotide separates consecutive CpGs, X.sub.1 is adenine, guanine, or thymine and X.sub.2 is cytosine, thymine, or adenine.

100. The method of claim 99, wherein the CpG-S motif is selected from the group consisting of GACGTT, AGCGTT, AACGCT, GTCGTT and AACGAT.

101. The method of claim 99, wherein the CpG-S motif comprises GTCGYT or TGACGTT.

102. The method of claim 99, wherein the CpG-S motif comprises TGTCGYT.

103. The method of claim 99, wherein the CpG-S motif comprises TCCATGTCGTTCCTGTCGTT (SEQ ID NO:1).

104. The method of claim 99, wherein the CpG-S motif comprises TCCTGACGTTCCTGACGTT (SEQ ID NO:2).

105. The method of claim 99, wherein the CpG-S motif comprises TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:3).

106. The method of claim 99, wherein the CpG-S motif comprises TCAACGTT.

107. The method of claim 85, wherein the antigen is a bacterial antigen.

108. The method of claim 85, wherein the antigen is derived from a parasite.

109. The method of claim 85, further comprising administering an antigen to the subject.